PROTOCOL TO PRODUCTION OF [6xHis] - GFP

1. Make a pre-inoculation of Rosetta :: pGFP(pET28a) of 10 mL LB containing 10 µL of Kanamycin (100 mg/mL) and 10 µL of cloramphenicol (35 mg/mL). Incubate overnight at 37°C under 200 rpm.

2. Add to an Erlenmeyer flask of 500 mL containing 100 mL of LB supplemented with previously referred antibiotics (ratio of 1 µL/mL of medium), about 5-10 mL of pre-inoculation. Incubate at 37ºC under 200 rpm until optical density at 600nm of 0.5-0.6 (Near to 1,5-2h)

3. Collect a sample of 1 mL (Non Induced), centrifuge for 5 min at 10,000 rpm. Eliminate supernatant and freeze pellet. Add 200 µL of 200 mM IPTG.

4. Cultivate at 37°C under 200 rpm for 4h. Collect a sample like in step 3 (now it will represent Induced sample)

5. Centrifuge for 5 min at 10,000 rpm at 4°C. Ressuspend pellet, in ice bath, in 10 mL of ice-cold lysis buffer without imidazole

6. Lysing cells by sonication (cycle On: 59,9s; Off: 30,0 s; total time: 10 min) with 24% of total amplitude. Tube should remains in ice bath all time, to ensure correct temperature along process.

7. Centrifuge lysate for 10 min at 20,020 xg at 4°C

8. Filter supernatant at 0,450 µm and purify via IMAC.